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NUCLEAR CHANGES IN EUROPEAN BLACK PINE SEEDLINGS CAUSED BY GROWTH REGULATORS

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Cytogenetic effects caused by two synthetic auxins: 1--naphtaleneacetic acid (NAA) and indole-3-butyric acid (IBA) as well as by synthetic cytokinin benzyladenine (BA), on root tip cells of ten-day-old European black pine (*Pinus nigra* Arn.) seedlings were observed. The results showed that auxins induced stronger cytogenetic effects than cytokinin. After treatment with auxins the mitotic activity and the corresponding number of chromosomal aberrations increased in parallel to the concentrations used. Both auxins, NAA and IBA, induced almost the same types of nuclear changes such as spindle failure, chromosome fragmentation, laggards and polyploidy. NAA induced a higher frequency of chromosomal changes than IBA. Also, the recovery period after the application of NAA was much slower as compared to that of IBA. Cytokinin BA showed the opposite effect. After treatment with the highest concentration the mitotic activity was very similar to the control, but after the recovery period the mitotic index increased significantly. This was also the case with a number of aberrant cells.

Introduction

A number of factors, chemical and physical, contribute to chromosome instability in cells cultured *in vitro* as well as in tissues and plants regenerated from them (Bayliss 1980, Papeš et al. 1983). The successful establishment of plant cell and tissues cultured *in vitro* depends mostly on composition of the medium, growth regulator concentrations and culture conditions such as temperature, light and photoperiod.

It is well known that in cultured tissues (Bayliss 1980) plant growth regulators, which induce proliferation, at the same time cause cytogenetic changes. Plants regenerated from callus or cells in suspension show genetic and phenotypic variabilities (Larkin and Scowcroft 1981), instability of chromosome structure and variation in chromosome number as reflected by polyploidy and aneuploidy (Bennici and D'Amato 1978, D'Amato 1985). Plant growth regulators control the cell division cycle at the molecular level (Houssa et al. 1990) as specific triggers in one or several steps of cell division (Fosket et al. 1977). In some species triggering of mitosis of differentiated cells may depend on a correct balance of exogenous auxins and cytokinins (Cionini et al. 1978).

In this study we investigated the effect of three synthetic growth regulators frequently used in tissue culture of gymnosperms (Kolevska-Pletikapić et al. 1983) on root tips of European black pine seedlings.

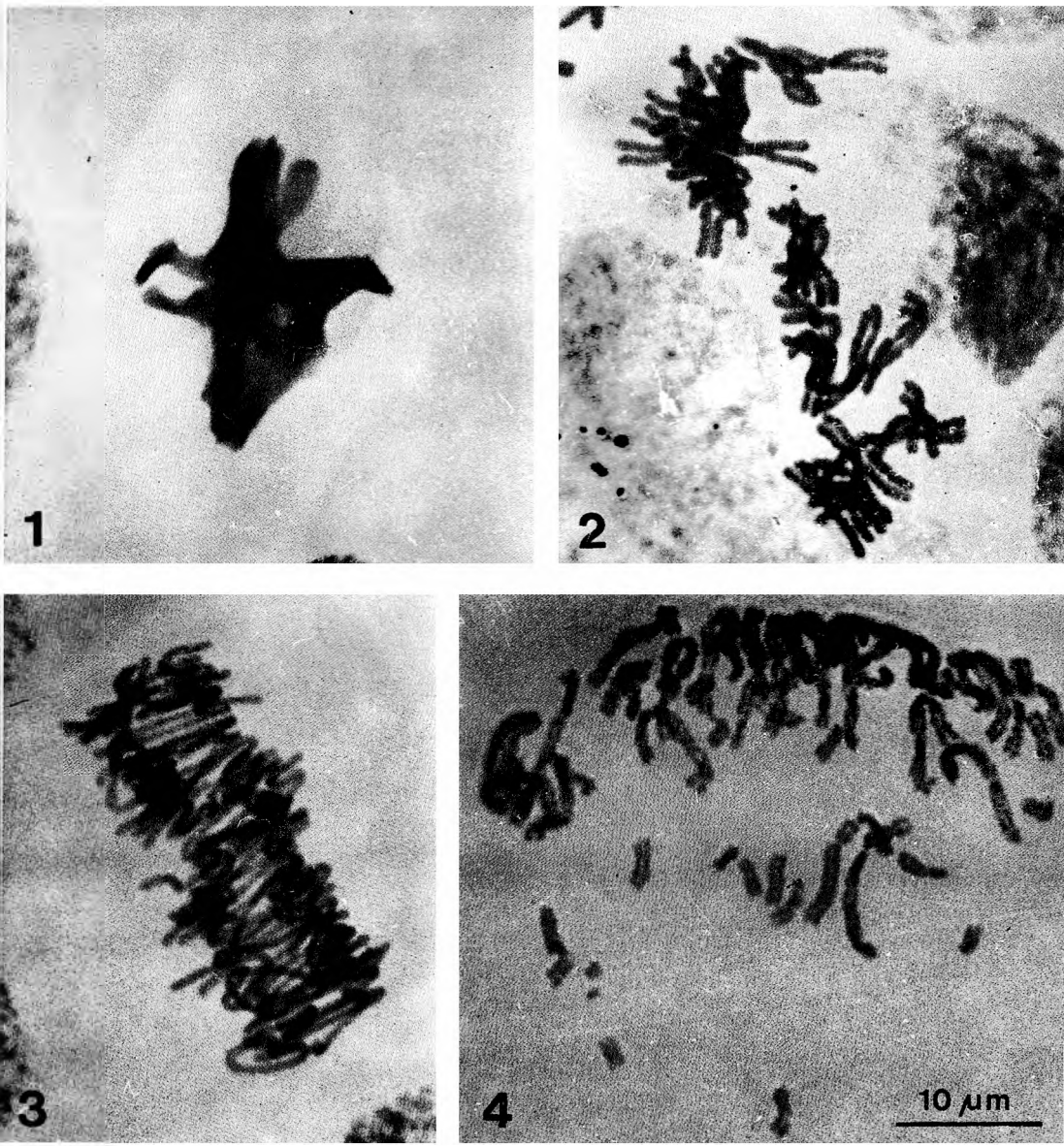
Materials and Methods

Black pine (*Pinus nigra* Arn.) seeds from the Institute for Forest Research, Jastrebarsko, were germinated in petri dishes on moist filter paper at room temperature. After ten days the seedlings were treated with various concentrations of two synthetic ayxins, 1-naphtaleneacetic acid (NAA) and indole-3-butyric acid (IBA), and synthetic cytokinin, benzyladenine (BA). The concentrations used varied from 10^{-3} to 10^{-7} M. The treatment time was 6 h with a period of 7 h recovery in distilled water. Seeds germinated in tap water were used as control (Fiskesjö 1988). For each concentration and the corresponding control at least 20 seeds were used. For microscopic studies root tips were cut and fixed in ethanolacetic acid (3:1) for at least 24 h. Slides were prepared using Feulgen squash technique (Sharma and Sharma 1972). Permanent slides were prepared using liquid carbon dioxide and after drying for 1 day the tissue was mounted in Euparal. In order to analyse the mitotic activity and the number of chromosomal aberrations 1500 cells from 20 root tips were scored per concentration and its corresponding control.

Results

1. *Effects of auxins*

Table 1 shows the mitotic activity and the total chromosome aberrations in black pine root tips after 6 h treatment with NAA and IBA. The mitotic activity decreased with the concentration used, while the mitotic activity of the control was lower than that of the treatment with the lowest concentration of NAA and IBA. The number of dividing cells after the recovery period increased slightly with concentration (Tab. 2). The highest number of aberrant cells was observed after treatment with the highest concentration (10^{-3} M) of each of the auxins (Tab. 1). However, the number of total chromosomal aberrations decreased with the concentration parallel to the mitotic activity. After the recovery period the amount of the total chromosomal aberrations was lower, and also decreased with the concentrations (Tab. 2).



Figs. 1—4. Chromosomal aberrations in root tip cells of *Pinus nigra* Arn. after 6 h treatment with NAA, IBA and BA as well as after a 7 h recovery period.

1. Chromosome stickiness and clumping chromation.
2. c-mitosis, metaphase.
3. Polyploid cell in anaphase as a result of c-mitosis.
4. Chromosome centric and acentric fragments in late prophase.

Table 1. Effects of different concentrations of NAA, IBA and BA on the mitotic activity and frequency of chromosomal changes in root tip cells of a ten-day-old black pine seedlings.

Growth regulator	Concentration (M)	No of cells examined	M. I. (%)	Total aberrant cells (%)
NAA	10^{-3}	1500	24.6	5.8
	10^{-4}	1500	17.6	4.2
	10^{-5}	1500	14.5	1.4
	10^{-6}	1500	10.6	1.2
	10^{-7}	1500	8.5	0.1
IBA	10^{-3}	1500	24.9	7.2
	10^{-4}	1500	14.7	2.3
	10^{-5}	1500	17.8	1.7
	10^{-6}	1500	14.6	0.8
	10^{-7}	1500	11.2	0
BA	10^{-3}	1500	9.0	0
	10^{-4}	1500	15.5	1.0
	10^{-5}	1500	15.0	0.3
	10^{-6}	1500	13.9	0.3
	10^{-7}	1500	13.9	0.3
Control		2000	9.5	0

Table 2. Mitotic activity and total chromosomal aberrations after a 7 h recovery period.

Growth regulator	Concentrations (M)	No. of cells examined	M. I. (%)	Total aberrant cells (%)
NAA	10^{-3}	1500	29.7	3.9
	10^{-4}	1500	17.4	2.8
	10^{-5}	1500	15.0	0.6
	10^{-6}	1500	19.8	0.6
	10^{-7}	1500	11.8	0.2
IBA	10^{-3}	1500	26.3	3.1
	10^{-4}	1500	22.4	0.2
	10^{-5}	1500	22.4	0.1
	10^{-6}	1500	17.3	0
	10^{-7}	1500	12.7	0
BA	10^{-3}	1500	18.0	0.7
	10^{-4}	1500	15.5	0.3
	10^{-5}	1500	20.2	0.2
	10^{-6}	1500	17.4	0.1
	10^{-7}	1500	15.5	0.2
Control		2000	9.5	0

Both synthetic auxins induced the same types of nuclear changes which were caused by hormonal effects either on the spindle or on chromosomal organization. The most prominent spindle abnormalities were c-mitoses (Fig. 2) and disturbed anaphase. Polyploidy was also observed, after a treatment with lower concentrations of NAA and IBA (10^{-6} and 10^{-7} M) and after a recovery period (Fig. 3). Chromosome fragmentation (Fig. 4), laggards in anaphase and telophase, anaphase bridges and micronuclei were observed as well. Stickiness was very frequent and it accompanied all the instabilities of spindle and chromosomal organization (Fig. 1).

2. *Effects of cytokinin*

After treatment with BA, the mitotic activity was lower than after treatment with auxins. Mitotic activity was similar to that of the control (Tab. 1). However, after the recovery period the mitotic activity increased significantly, especially after treatment with the highest concentration (Tab. 2). The number of the total chromosomal aberrations was lower than after the treatment with auxins, although it increased slightly after the recovery period (Tabs. 1 and 2), especially after the treatment with the highest concentration. Laggards in anaphase and telophase were noticed after the recovery period. After treatment with BA micronuclei and anaphase bridges were also observed, while polyploid cells were present only in a small number after the recovery period.

Discussion

Auxins

Data obtained in treatments with NAA and IBA show similarities in mitotic activity (Tab. 1). The mitotic index decreased parallel with the concentrations of either NAA or IBA. But after the treatment with the lowest concentration of NAA or IBA (10^{-7} M) the mitotic activity was higher than in the control. The synthetic auxins NAA and IBA stimulated cell division, which was dose-dependent. After the treatment with the highest concentrations of NAA or IBA (10^{-3} M) the mitotic activity was 2.5 × higher than in their respective controls. The explanation of these results could be the fact that in tissue culture auxins are almost invariably required to promote the initial growth of the meristem, so they stimulate both cell growth and division. Similar results were obtained after the recovery period (Tab. 2). The total chromosomal aberrations occurred in a dose-dependent manner, that is, the highest number of aberrant cells was observed after treatment with the highest concentration of either NAA or IBA, of which NAA had the stronger effect. NAA and IBA induced almost the same types of nuclear changes. The most prominent spindle abnormalities were the c-mitosis and disturbed anaphases observed also in shallot root tips after treatment with 2,4-D (Pavlica et al. 1991). It is well known that synthetic auxins like NAA and 2,4-D used in herbicide concentrations (> 50 μg/ml), can induce spindle failure and other mitotic abnormalities in intact plants (Bayliss 1980). Chromosomal fragmentation and laggards at anaphase were also observed. Spindle failure and chromosome laggards may influence the process of polyploidization (D'Amato 1985), as we also noticed in our experiments.

Cytokinins

After treatment with BA the mitotic activity was lower than that following treatment with auxins. Since cytokinin synthesis takes place in meristematic tissue, mainly in root-tip cells (Short and Torrey 1972), and the administration of BA on intact roots decreased the mitotic activity, this may have been the consequence of interaction between exogenous and endogenous cytokinins. After the recovery period we noticed a significant increase of mitotic activity, probably because of a delay in cytokinin activity. We noticed the same trend in the number of aberrant cells. There were laggards, bridges and micronuclei, a sign of chromosomal breakage and elimination (Ogura 1982). The mechanism of induction of chromosomal aberration could be a consequence of the chemical structure of BA, a derivative of purine, allowing for its possible integration into DNA. Also, plant cells contain a protein which appears to be involved in the initiation of DNA replication (Houssa et al. 1990) and in the regulation of protein synthesis (Maas and Klambt 1977). The question thus is, whether BA acts directly or indirectly on nucleic acids. From our results we conclude that, in addition to the well known test-objects *Allium cepa* and *Vicia faba*, European black pine is also a very useful test-plant for detection of cytotoxicity.

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SAŽETAK

KROMOSOMSKE PROMJENE U KLIJANACA CRNOG BORA IZAZVANE REGULATORIMA RASTENJA

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Istraženo je djelovanje dva sintetska auksina: 1-naftalenoctene kiseline (NAA) i indolil-3-maslačne kiseline (IBA) i sintetskog citokinina benziladenina (BA) na meristemske stanice vrška korjenčića 10 dana starih klijanaca crnog bora (*Pinus nigra* Arn.). Auksini imaju snažnije citogenetsko djelovanje na genom meristemskih stanica od citokinina. Nakon tretmana auksinima mitotska aktivnost stanica je porasla paralelno s upotrijebljenom koncentracijom. Broj aberantnih stanica također se povećavao s koncentracijom auksina. Oba auksina induciraju gotovo iste tipove promjena na razini diobenog vretena i na razini kromosoma. Sintetski citokinin BA je pokazao suprotno djelovanje. Nakon tretmana s najvećom koncentracijom mitotska aktivnost bila je vrlo slična onoj u kontrolnom uzorku. Međutim nakon oporavka značajno je porasla, a također je porastao i broj kromosomskih aberacija.

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